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13. ABSTRACT (Maximum 200 words) Progress was made in a number of areas related to the role of the central noradrenergic system in the behavioral effects of stress. First we established that norepinephrine mediates the persistent anxiety after stress as others have found in various learning paradigms thus linking studies of stress to studies of learned anxiety. Second we showed that a likely factor in this effect is the stimulation of the gene, c-fos, which we found to be stimulated throughout the brain by the noradrenergic system during stress. Third we discovered a new phenomenon caused by stress involving a transient reduction in arousal which causes animals to lose caution in dangerous situations and may be related to a temporary desensitization of the brain beta-1 adrenoceptor. Fourth we found that repeated stress in mice causes a progressive decline in nocturnal activity and feeding thus establishing a new animal model of depression which will facilitate studies of the role of the noradrenergic system in behavioral depression.			
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Title: Measurement and regulation of central noradrenergic receptors

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Final Technical Progress Report

Progress was made in a number of areas related to the role of the central noradrenergic system in the behavioral effects of stress. First we established that norepinephrine mediates the persistent anxiety after stress as others have found in various learning paradigms thus linking studies of stress to studies of learned anxiety. Second we showed that a likely factor in this effect is the stimulation of the gene, c-fos, which we found to be stimulated throughout the brain by the noradrenergic system during stress. Third we discovered a new phenomenon caused by stress involving a transient reduction in arousal which causes animals to lose caution in dangerous situations and may be related to a temporary desensitization of the brain beta-1 adrenoceptor. Fourth we found that repeated stress in mice causes a progressive decline in nocturnal activity and feeding thus establishing a new animal model of depression which will facilitate studies of the role of the noradrenergic system in behavioral depression.

Studies, Results and Significance

In the first aspect of the work we examined the role of brain norepinephrine (NE) in mediating the persistent anxiety caused by stress in the rat. Previous studies by others had suggested that NE is involved in anxiety learned to a brief aversive stimulus but it had not been clear if this also holds for anxiety after typical laboratory stresses which are more prolonged. To study this we subjected rats to a standard laboratory stress, 2 hr of immobilization, and tested them for anxiety 24 hrs later in an open field or a plus maze. The stress produced a significant inhibition of open field and plus maze activity in agreement with its anxiogenic properties. Pretreatment (i.p.) of rats prior to stress with the beta-1 antagonist, betaxolol, or the alpha-1 antagonist, prazosin, significantly attenuated the anxiogenic

effects of the stress. A beta-2 antagonist, ICI118,551, an alpha-2 antagonist (yohimbine), a dopaminergic D1/D2 antagonist (fluphenazine) and a 5HT2 antagonist (ketanserin) were all ineffective in producing this change. The effect of betaxolol could not be explained on the basis of sedative effects. It is concluded that adrenergic mechanisms involving beta-1 and alpha-1 receptors are necessary for stress to induce persistent anxiety in the rat. This finding (Stone and Quartermain, in preparation) links two major fields of research, traditional stress research and the neuropharmacology of learning which has shown a major noradrenergic involvement in various types of learning and suggests that a good part of the anxiogenic effect of stress is learned. This finding will have major implications for future studies on the mechanism of stress-induced anxiety.

In the second phase of the research we obtained evidence that the noradrenergic system may produce the above persistent change by stimulating immediate early genes (IEGs) during stress. We had shown in previous research that the noradrenergic system activates at least 5 IEGs in the brain (Bing et al., 1991). We had also shown that stress activates these same genes but we had not yet determined whether the noradrenergic system was responsible for the stress-induced responses. To accomplish this we pretreated mice with various blocking agents and subjected the animals to 1 hr of immobilization stress, perfusing the animal for c-fos immunohistochemistry 2 hr after the end of the stress (Stone and Zhang, submitted). The results indicated that prazosin blocked the fos response in 10/12 telencephalic, 2/6 diencephalic and 3/5 brainstem regions and that betaxolol blocked it in 6/12 telencephalic, 1/6 diencephalic and 0/5 brainstem regions. ICI118551, fluphenazine, WAY100135 (selective 5HT1A antagonist) and ketanserin did not have any general effects on the response. These results indicate that the noradrenergic system is the major factor producing a fos response throughout the brain during immobilization stress in the mouse. This response may be involved in various persistent behavioral actions of stress including the anxiety response studied above.

The third aspect of the work has dealt with a new phenomenon, reduced arousal after stress in mice. This phenomenon was discovered in the course of our attempts to produce an animal model of depression in mice. It was found that various forms of stress produced a transient 3 hr reduction in locomotor and swimming activity and an increase in grooming behavior in the mouse (Stone et al., In press). The same behavioral profile could be produced by giving the mice central nervous system depressants (Bainbridge and Greenwood, 1971). To test if the animal's arousal was reduced we examined the effects of stress (immobilization) on arousal from anesthesia. A marked prolongation of the sleeping time to both halothane and hexobarbital was found in the stressed animals (Stone, Manavalan & Quartermain, In preparation). To determine whether the effect would have ethological consequences for the animal we assessed the effects of stress on behavior in an environment in which mice are normally extremely cautious, entry into a brightly lit open field. It was found that the

stressed mice showed a total lack of caution, entering into the center of the field immediately as compared to the nonstressed animals who entered slowly (circa 30 min) and stayed close to the walls (Quartermain et al., submitted). To study the relation of the effect to the noradrenergic system we treated mice with various monoaminergic receptor blocking agents and examined their behavioral effects. It was found that the nonselective beta blocker, propranolol, and the selective beta-1 blocker, betaxolol both produced identical effects as stress on locomotor, swimming and grooming behavior and on anesthesia sleeping time (Stone et al., In press). This was not seen with a peripheral beta-1 blocker, atenolol, nor with the beta-2 blocker, ICI118551, fluphenazine, WAY100135 and ketanserin. These findings suggest that stress produces a transient interference with beta-1 neurotransmission in the brain which reduces arousal and may have important survival consequences for the animal. The reduced beta-1 neurotransmission may be the result of a temporary desensitization of beta-1 receptors or a depletion of glycogen which is under beta-1 receptor control in the brain.

The fourth phase of the work has involved studies of the effect of stress on nocturnal activity in mice with the aim of developing an animal model of depression. Previous work on animal models of depression in rodents have generally utilized studies of the animals during the day when they are normally asleep. Studies of activity at night when the animals are normally active may have greater validity to depression. To investigate this we have assayed three measures of activity at night in mice, wheel running, total movement (jiggle cage) and feeding behavior (bar pressing for food reinforcement) after a mild stress, restraint in a tube. We have found that as mice are exposed to this stress daily their activity and bar pressing for food progressively declines to a low of approximately 50% at 4 days of stress and returns to normal when the daily stressors are stopped (Stone and Quartermain, In preparation). This behavioral change does not appear to result from learned anxiety since it occurs in the absence of any cues present during the stress. As such the behavior may be a relatively purer form of depression than the various daytime models now in use which usually entail some contextual learning. Studies are currently in progress to test the effects of antidepressants and adrenergic receptor blocking agents on these behavioral changes.

Publications 2/23/94-pres.

a) Papers

Stone, E.A., Najimi, M. & Quartermain, D. Potentiation by propranolol of stress-induced changes in passive avoidance and defensive withdrawal. Pharmacol Biochem. Behav, In press.

Stone, E.A., Manavalan, S.J., Zhang, Y. & Quartermain, D. Beta adrenoceptor blockade mimics effects of stress on motor activity

in mice.. Neuropsychopharmacology, In press.

Watanabe, Y., Stone, E.A. & McEwen, B.S. Induction and habituation of c-fos and zif/268 by acute and repeated stressors. Neuroreport 5:1321-24, 1994.

Stone, E.A., Zhang, Y. & Carr, K.D. Massive activation of c-fos in forebrain after mechanical stimulation of the locus coeruleus. Brain Res. Bull. 36:77-80, 1995

Quartermain, D., Stone, E.A. & Charbonneau, G. Acute stress disrupts risk assessment behavior in mice. Submitted.

Stone, E.A. & Zhang, Y. Inhibition of the c-fos response to stress in mouse brain by adrenoceptor antagonists. Submitted.

Stone, E.A., Manavalan, J.S. & Quartermain, D. Effect of stress and beta-1 adrenoceptor blockade on arousal from anesthesia in mice. Submitted.

b) Abstracts

Quartermain, D., Stone, E.A. & Charbeneau, G. Acute stress disrupts defensive behavior in mice. Abstr. Soc. Neurosci. 20:1445, 1994.

Manavalan, J.S., Najimi, M., Stone, E.A. & Quartermain, D. Effect of propranolol on stress-induced changes in passive avoidance and open field emergence tests. Abstr. Soc. Neurosci. 20:1445, 1994.

Stone, E.A., Quartermain, D., Manavalan, J.S. & Zhang, Y. Beta-1 adrenoceptor blockade mimics the behavioral effects of acute stress in mice. Abstr. Soc. Neurosci. 20:1444, 1994.

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Other literature

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Bing, G., D. Filer, J.C. Miller and E.A. Stone. Noradrenergic activation of immediate early genes in rat cerebral cortex. Mol. Brain Res. 11:43-46, 1991.